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(54) Title: DEVICE AND METHOD FOR PREPARING A SAMPLE FOR ANALYSIS

(57) Abstract: The invention provides apparatus and methods for dividing a sample, especially a liquid sample, into a plurality of subsamples without the need for pipetting or for other manual operations. The invention is expecially useful to isolate entities suspected to be present in a sample for individual analysis.

DEVICE AND METHOD FOR PREPARING A SAMPLE FOR ANALYSIS

Field of the Invention

The invention provides devices and methods for dividing a sample into subsamples.

Background of the Invention

It is often desirable to detect the presence in a complex biological sample of one or more molecules present in low frequency in the sample compared to another, more common molecule. For example, the detection of mutations in oncogenes or tumor suppressor genes or loss of heterozygosity (LOH) in tumor suppressor genes at an early stage of oncogenesis is useful for early diagnosis of cancer. Such detection preferably is done in a specimen obtained through non-invasive, or minimally invasive means. Typical specimens include stool, sputum, and other specimens that have a complex mixture of cellular components. DNA from cells having mutations indicative of early-stage cancer are present in such specimens in low frequency with respect to wild-type DNA. Detection of mutant DNA in a sample using conventional techniques is often difficult because the signal associated with such low-frequency DNA is undetectable even if the target DNA is present in the specimen, or in a sample derived from the specimen.

Similar problems exist in the detection of other low-frequency molecular species. For example, the detection of the relative amounts of high- and low- expression proteins may be undetectable over highly-expressed protein. A similar situation exists in detecting RNA, and other cellular components.

With the advent of the polymerase chain reaction (PCR), detection of nucleic acids became routine, as the PCR allowed one to amplify vast quantities of a molecule of interest. However, PCR alone does not completely solve the problem of detecting low frequency molecules in a heterogeneous sample. For example, PCR will amplify a low frequency nucleic acid if the PCR primers hybridize to the low frequency sequence. If a low frequency target amplicon is not amplified in the first rounds of PCR, the probability that it will be amplifiable and detectable in successive rounds decreases with each round. Thus, attention must be paid to the amount of material presented to the PCR, the efficiency of the PCR, and the representative nature of the input sample. See, e.g. U.S. Patent Application No. 60/109,567, incorporated by reference herein.

Digital PCR is one method that has been developed to overcome the limitations of using traditional PCR techniques to detect low-frequency molecular events. See, Vogelstein et al., Proc. Natl. Sci., 96: 9236-9241 (1999), incorporated by reference herein. Digital PCR results in amplification of single target molecules to produce a

digital signal that is especially good for detecting low-frequency molecules. Specifically, digital PCR operates by taking a sample, diluting it, and dividing it into tens of thousands of subsamples each in its own well so that most sub-samples contain either zero or one target molecule(s), and very rarely do sub-samples contain more than one target. The subsamples are then amplified and detected individually using PCR. PCR performed on subsamples results in pure amplification of a single target molecule, whether mutant or wild-type. Consequently, each well or discrete result in the PCR is homogeneous replicate of the original single starting molecule. This makes possible the determination of whether that starting molecule was mutant or wild-type. The entire sample can then be characterized simply by counting the number of mutant and wild-type wells and taking the ratio.

Digital PCR is limited in its ability to detect low-frequency molecules. Specifically, there is a problem in distributing or fractionating sample among numerous wells in order to conduct digital PCR to detect a mutant nucleic acid present in a sample in low frequency (i.e., in a sample containing a high mutant:wild-type ratio). Similar problems exist if the sample has been pooled from different patients (i.e., the target nucleic acid exists in the pooled sample in low frequency compared to other nucleic acids).

Accordingly, there is a need in the art for methods of distributing a sample into subsamples without the need for pipetting or other physical manipulations of the sample in order to allow efficient detection of low-frequency molecular events.

Summary of the Invention

The present invention provides devices and methods for conducting parallel reactions on material in a liquid sample. Such material may be a molecule or molecules (e.g., a nucleic acid, a protein, a carbohydrate, etc.), cellular components, by-products of cellular metabolism, cellular debris, whole cells, or acellular molecules (e.g., minerals, metals, etc.). Thus, as referred to herein the terms "material" or "entity" refer to any cell, cellular component, or noncellular substrate the detection and/or reaction of which is desired. The invention comprises a microfluidic device and methods for its use which allow advantageous sample fractionation. According to the invention, a sample fractionation device comprises an inlet for application of a liquid sample, and a plurality of channels, vents, restrictions and other features configured to create vacuum breaks within the channels to isolate discrete portions of a sample. The invention solves the problem of sample fractionation (distribution of a single sample into a multiplicity of subsamples), especially when it is desired to isolate low-frequency material thought to be present in the sample or when the required subsample volume is too small to allow effective serial dispensing using physical processes such as pipetting.

In a preferred embodiment, a sample is divided into a plurality of subsamples in a stochastic process that results in the discrete distribution of material for analysis and/or detection. According to the invention, a liquid sample is forced into a sample flow channel (or simultaneously into several channels in parallel). The sample flow channel comprises a series of bends. Alternating vents and valves are placed at the bends, such that when the vents are opened a series of vacuum breaks at the bends isolate portions of the liquid sample in the elongate areas of the channels between the bends, turning a single, continuous sample into a series of discrete, isolated subsamples.

In a particularly preferred embodiment, liquid sample is forced, via a primary sample flow channel (exemplified below as a "main charging channel"), into a series of secondary channels or wells (referred to below as "fractionation channels"), each having at least one pressure-actuated valve As described in detail below, the pressure source that induces sample flow into the channels may also open the valves at a higher pressure. Increased pressure causes sample in the primary sample flow channel to be purged into a purge channel through a hydrophobic pressure-actuated valve which joins the primary sample flow channel to the purge channel. The valve is configured to open at the increased pressure. The purge channel may be configured similarly to the fractionation channels to perform fractionation on the purged portion of the sample, thereby preserving the purged portion of the sample for further analysis. Sample is prevented from flowing out of the fractionation and purge channels by other hydrophobic valves which actuate at an even higher pressure, thereby dispensing fractionated subsamples into discrete chambers or wells.

The dispensing of sub-samples from the fractionation channels is permitted by vents in the fractionation channels which create a series of vacuum breaks. These vents introduce a gas volume ("bubbles") to replace the void left by the flowing liquid at multiple points in the fractionation channels, thereby isolating discrete subsamples between the bubbles in the fractionation channels. The resulting subsamples are then deposited through a hydrophobic valve into wells or chambers for analysis.

A preferred device of the invention comprises a reservoir for holding a sample. The reservoir is in fluid communication with a fractionation channel into which a portion of the sample flows. The fractionation channel comprises a plurality of apertures for deposition of subsamples for analysis. The fractionation channel is also in communication with a vent for fractionating sample as described below. In operation, sample is deposited into the reservoir and is forced into the fractionation channel where hydrophobic vents create a series of vacuum breaks to fractionate the sample into subsamples. The subsamples are then deposited for analysis into wells or chambers in communication with the fractionation channels

A preferred embodiment is shown in Figure 1. An exemplary device of the invention comprises a main charging channel 1 in fluid communication with a reservoir 4. The main charging channel 1 is also in fluid communication with a plurality of fractionation channels 5, each in communication with a vent 7. Finally, the charging channel 1 is in communication with at least one purge channel 9.

In operation, sample is placed into the reservoir 4 and is then forced into the main charging channel 1 and distributed into each fractionation channel 5. Sample is then evacuated from the main charging channel 1 into the purge channel 9. Vents 7 in communication with each fractionation channel 5 create vacuum breaks in the fractionation channels (e.g. when a spinning force is applied in a direction radiating away from the vent), thereby permitting fluid flow out of the fractionation channels 5. The result is a plurality of subsamples in the fractionation channel, each separated by gas introduced by the vents 7 during the deposition of the subsamples into reaction chambers or wells. Devices of the invention may have multiple charging channels to facilitate movement of sample into fractionation channels.

In a preferred device of the invention, fractionation channels 5 and fractionating purge channel 9 comprise angled bends, with alternating bends having either a hydrophobic valve or a vent, such that, upon operation of the device, sample accumulates in the elongate portions of the channel between each bend. A preferred configuration is a "zig-zag" configuration as shown in Figure 1. Also in a preferred embodiment, channels comprise a hydrophobic coating . In a further preferred embodiment, fractionation channels 5 and purge channel 9 comprise valves for distribution of subsamples into analysis chambers. According to the invention, valves may be mechanical, but are preferably pressure-actuated extensions of the channel that narrow to prevent fluid flow through the valve during sample accumulation (charging), but which allow subsample flow upon application of increased pressure (over that which is used to charge the channels). In a highly-preferred embodiment, valves are pressure-actuated hydrophobic valves or neck-down channels, such as those shown in Figure 2. Channels may emanate from a central or main charging channels as shown in Figure 1, or they may emanate from a central inlet (e.g., in a disk conformation), or they may take any other shape that is compatible with the invention as described herein.

In a highly-preferred embodiment, the invention provides a microfluidic sample separation device comprising a sample inlet or reservoir in fluid communication with a first channel which is, in turn, in fluid communication with a plurality of second (sample fractionation) channels in a zig-zag configuration for subsample accumulation and a third channel for excess sample contained in the first channel after the second channels have been completely filled. Each of the second channels comprises a hydrophobic

coating and a series of alternating vents and pressure-actuated, hydrophobic valves in communication with wells for subsample deposition. The third channel comprises a pressure-activated, hydrophobic valve that is activated at a lower pressure than the valves contained in the second channels. Also in a highly-preferred embodiment, channels are etched into a substrate, such as silicon, plastic, or a polymer suitable to conduct samples and to provide venting and valve functionality. Preferred substrates include solid Teflon® ,Teflon®-coated polycarbonate, etched copper, or any etched, stamped or molded substrate which can be made hydrophobic. In a preferred embodiment, each well comprises a purge vent which allows air to escape as fluid enters the well. This additional valve is activated at a very high pressure so that liquid does not flow out of it.

Also in a preferred embodiment, vents for creating a vacuum break in fractionation channels are supplied on a planar sheet of material having pores of sufficient size for venting. Preferably such material is a Gore-tex or Zitex coating, or similar substrate with pores sufficiently small to allow the passage of gas (e.g., air), but not of liquid (e.g., sample). In a preferred embodiment, such coating is placed over a flat substrate into which charging, fractionation, and purge channels are etched. A subsample receptacle comprising a plurality of wells for receiving subsample (e.g., a microtiter plate) is place on the underside of the device such that each well communicates uniquely with a valve on the fractionation channels. The device is preferably clamped together by, for example, a friction fit or other engagement, so that the sides of the device are sealed against leakage of sample. Once sample is fractionated into subsamples, the subsamples may be deposited into wells for analysis via the valves in the fractionation channels.

In another preferred embodiment, centrifugal force is used to load sample into the various channels. Thus, in an alternative embodiment, valves in devices of the invention may have different pressure actuation points by, for example, varying their diameter. Varying the actuation pressure of valves accommodates uniform filling of fractionation channels at uniform RPM, even when the channels are spaced at different distances from the sample reservoir. Also in an alternative embodiment, vents for use in a device of the invention are contained in a lid that fixes to the surface of a plate onto which channels are etched. Preferably, such a lid comprises venting apertures having a diameter smaller than that of the valves associated with fractionation channels.

The dimensions of channels, valves, vents, and other components of devices of the invention are determined based upon the surface tension of the liquid to be fractionated and the hydrophobicity of the material from which the device is made. One of ordinary skill in the art understands that devices according to the invention are dimensioned and configured according to the needs and expectations of the individual

investigator based upon physical chemical principles. Similarly, the number of fractionation channels, valves, vents, and other components may be varied at the convenience of the user. Thus, without limitation, a preferred device of the invention comprises a main charging channel that is about 500 um wide and about 800 um deep, valves in fractionation channels that are about 50 um square, and have a length of about 250 um. Fractionation channels are about 500 um wide and about 500 um deep. A preferred purge valve is about 500 um long and about 135 um square. Devices of the invention are made to accommodate the largest microtiter plates, thus allowing fractionation of a sample into extremely small volumes such that at least some subsamples contain a single entity (e.g., a single nucleic acid fragment). Preferably, however, subsamples are deposited directly into wells attached to the fractionation plate.

Methods of the invention comprise dividing a sample into subsamples using a device of the invention. Preferred methods comprise introducing a sample into a channel, purging sample from at least a portion of the channel, and venting a portion of the channel thereby to create a vacuum break in the channel in order to isolate a subsample for analysis. Also in a preferred embodiment, methods comprise introducing a sample to a first channel, forcing sample from the first channel to a second channel, purging sample from the first channel, and creating one or more vacuum breaks in the second channel, thereby to isolate subsamples. In a particularly-preferred embodiment, sample is purged from the first channel into a third channel that is separated from the second channel. Also in a preferred embodiment, sample is forced into a plurality of second channels, each comprising at least one vent for creation of a vacuum break. The second channels also provide valves for dispensing subsamples into dispensing wells or other containers for identification or analysis. Preferred methods of the invention comprise the additional step of rotating the plate containing channels at sufficient velocity to create a centrifugal force sufficient to induce flow through the appropriate valves in order to distribute sample in the channels. Rotation is also a preferred way to force sample into second channels, and to activate venting for sample fractionation. Rotation may be accomplished by providing a force vector on the sample sufficient to impel the sample through the channels. For example, centrifugation may be used. Alternatively, sample may be distributed by vacuum, a syringe, wicking, simultaneous positive pressure at all the vents, and other methods known in the art.

Accordingly, preferred methods of the invention comprise applying a liquid sample to a device comprising a series of channels or capillaries having a configuration of valves and/or vents as described above; separating sample into subsamples; and analyzing the subsamples. In a preferred method, the sample is a biological sample, such as a tissue homogenate, body fluid, or body effluent. Such samples include, but

are not limited to, blood, pus, stool, aspirate, sputum, cerebrospinal fluid, lymph, saliva, semen, homogenates of tissue samples (whether diseased or not), and other bodily secretions, excretions, and the like. Methods of the invention isolate nucleic acids, proteins, carbohydrates, viruses, bacteria, cells, ligands, receptors, and other entities for analysis, identification, or other manipulation. Methods of the invention are also useful to isolate subsamples of non-biological material, including, but not limited to, minerals, metals, and the like.

In a highly-preferred embodiment, methods of the invention are used to isolate from a heterogeneous biological sample, a low-frequency molecule thought to be present in the sample. For example, preferred methods of the invention are useful to isolate a mutant nucleic acid fragment suspected to be in the sample. Methods of the invention isolate even a very low frequency mutant because such methods result in the binary distribution of sample molecules. Application of a sample to a device of the invention in a volume less than the volumetric capacity of the device allows, upon venting the device, isolation of subsamples, some of which contain a single molecule of interest. In the case of nucleic acids, methods of the invention are useful for preparing samples for digital PCR analysis, which is described in Voglestein, et al., Proc. Natl. Acad. Sci., 96: 9236-9241 (1999), incorporated by reference herein.

Regardless of the application to which they are put, devices and methods of the invention enable isolation, identification, analysis, or reaction of discrete entities present in even the most complex samples. The following detailed description of the invention provides a description of a preferred embodiment of the invention. The skilled artisan is aware of the broad range of applicability of the invention upon consideration of the following.

Description Of The Drawings

Figure 1 is a schematic diagram of channels and a reservoir etched into a device according to the invention.

Figure 2 is a schematic diagram of a neck-down valve for use in devices and methods of the invention.

Figure 3 shows posts on the underside of a device of the invention for depositing sample into a receptacle.

Figure 4 is a table representing a sample 360-well plate into which subsample has been deposited according to the invention.

Detailed Description Of The Invention

The present invention provides a microfluidic device for sample fractionation. Devices of the invention comprise combinations of channels for advantageous fractionation of liquid samples. The channels comprise alternative vents and valves, and are configured to allow induced vacuum breaks to isolate small subsamples from

even the smallest sample. As a result, devices and methods of the invention allow isolation in subsamples of single entities for identification, characterization and/or analysis. The examples provided below illustrate a preferred embodiment for practicing the invention. The skilled artisan appreciates that numerous additional embodiments and alternatives are within the scope of the invention disclosed herein.

Example 1

A preferred embodiment of the invention is shown in Figure 1. Sample is placed, via inlet 2 into sample reservoir 4 that is etched into fractionator plate. The reservoir 4 is in fluid communication with a main charging channel 1 which, in turn, is in fluid communication with a plurality of fractionation channels 5, and terminal purge channel 9. Each fractionation channel 5 comprises a series of bends and folds, shown as a "zig-zag" configuration in Figure 1. Alternating bends in the fractionation channels 5 terminate in a valve 6. Opposite bends terminate in a vent 7. The valves are hydrophobic pressure-actuated valves as shown in Figure 2. Each valve comprises a "neck-down" feature that prevents fluid flow in the neck under low pressure. Valves comprise a "neck-down" or tapered region that is about 250 um long and about 50 um square, terminating in a port for dispensing sample or terminating in a vented well. In the embodiment shown, fractionation channel valves terminate in hollow posts as shown in Figure 3, for mating with a receptacle, such as a microtiter plate, for distribution of subsamples for analysis. The terminal purge channel comprises an entrance valve that is activated at a higher pressure than that which is used to fill the fractionation channels but at a lower pressure than that used to empty the fractionation channels, or to dispense the samples.

Prior to its use, the plate described above is mated with a receptacle plate, preferably a microtiter plate, for receiving subsample via fractionation channel valves 6, and to a lid which seals the channels against leakage. In operation, the sealed device is subjected to centrifugation in order to distribute sample, and to create subsamples. A first spin distributes sample into the main charging channel 1 and into the fractionation channels 5. A second spin at higher RPM (thereby increasing pressure in the channels) purges the main charging channel 1. A final spin activates venting in the fractionation channels 5 and distributes sample via the valves to the receptacles (e.g., microtiter plate). Sample distribution can occur by any means that causes sample to be forced through the device at differential pressures. Centrifugation is preferred, however, because one is able, by choosing appropriate materials, spin angles, and RPM, to precisely control sample distribution and fractionation. The parameters described above may be varied according to the principles on which the invention is based. For example, the flow of sample through a device of the invention is controlled, *inter alia*, by

the physical chemical characteristics of this sample and the device. Channel width, as well as valve size, may be varied as a function of the hydrophobicity of the device and the surface tension of the liquid sample. It is these physical chemical properties that also determine the RPM appropriate for sample fractionation, purging, and dispensing. Accordingly, the skilled artisan can vary parameters of the invention either according to knowledge in the art or by empirical means. The following description applies to a device used to fractionate a typical biological sample that was prepared by homogenization.

Example 2:

Fractionation of a Biological Sample

A device of the invention was made by cutting a sample reservoir and channels in a fractionation plate in the pattern shown in Figure1. The plate itself was Teflon® and channels were cut using a 500 um endmill at 8000 RPM and -40° C to generate a main charging channel 1 having a width of about 500 um and a length of about 95 mm, and 23 fractionation channels 5, each having an overall length of about 350 mm. The purge valve 8, 135 um square and about 500um long, and dispense valves, 50 um square, were machined using laser ablation. Each fractionation channel comprised 16 valves (each 50 um square and 250 um long) The plate was attached via four 6 mm diameter by 25 mm long screws to a 384 well microtiter plate. To the face of the plate was attached a foam-backed porous Zitex® PTFE membrane that was 6mm thick. Venting was accomplished by placing a porous sheet, such as Gortex or Zitex, between the fractionation plate and the lid. The pores in such materials must be of a diameter less than these valves. In this embodiment of the invention, the porous sheet acts as a vent for the plate as a whole. On top of the foam-backed porous Zitex® PTFE membrane was a lid that was fixed to the device by the screws previously mentioned. The microtiter plate, fractionation plate, foam-backed porous Zitex® PTFE membrane and lid assembly was assembled. Sample was introduced to the inlet through a matching orifice in the lid and foam-backed porous Zitex® PTFE membrane. Once introduced, sample was housed in the reservoir of the fractionation plate. The entire assembly was then spun at 103 RPM for 4 minutes in order to distribute sample throughout the main charging channel and the fractionation channels in the fractionation plate. The assembly was then spun at 125 RPM for 4 minutes to purge the main charging channel into the purge channel. The assembly was then spun at 600 RPM for 10 minutes to activate the vent function in the foam-backed porous Zitex® PTFE membrane which sealed the fractionation channels, thereby creating a series of vacuum breaks in the fractionation channels. The series of vacuum breaks separates the sample into subsamples. A final spin forces subsamples into the wells of the microtiter plate for analysis. In an

alternative embodiment, the vents are incorporated into the fractionation plate along with the channels and hydrophobic valves.

Reagents for reacting components of the deposited subsample are then added to the assembly in the same manner as described above, and divided and dispersed as described above into the wells of the microtiter plate. Appropriate chemical reactions may then occur. A particular application of the invention is the preparation of samples for PCR. This embodiment is described below in Example 3.

If necessary, the hydrophobic valves incorporated into the plate may be dried between the fractionation of the sample and the subsequent fractionation of the reagents. Such drying may be necessary to maintain or restore the functionality of the hydrophobic valves, as a hydrophobic valve may be rendered less hydrophobic by the presence of liquid plating the walls of the valve.

Example 3:

In this example, a homogenate prepared from a biological specimen is used as the sample. A mutant nucleic acid suspected to be in the sample is isolated, and analyzed using digital PCR. In this case, a stool sample is homogenized in a buffer comprising 500 mm Tris, 16 mm EDTA, 10 mm NaCl, Ph 9.0, and a 1 ml aliquot is taken. For purposes of illustration and ease of calculation, it is assumed that the aliquot contains 150 wild type DNA molecules and a 3% mutant:wild type ratio. A device is prepared as described above using a 360 well microtiter plate as the receptacle for subsamples.

The 1 ml aliquot of sample is placed in the sample inlet of a sealed device as described above. The sealed device is centrifuged at 103 RPM for 4 minutes in order to load the main charging channel and the fractionation channels. The device is spun a second time for 4 minutes at 125 RPM to purge the main charging channel into the purge channel. A third spin causes subsamples of the original aliquot to be deposited into respective wells of a microtiter plate (see above).

Reagents for PCR, including Taq polymerase, appropriate primers, and buffers, are added to the sample inlet and the device is again subjected to the three centrifugation steps described above (RPM and spin time is the same as above). This causes PCR reagents to be deposited in the wells containing subsample. Standard PCR is then conducted.

The skilled artisan appreciates that numerous other reactions may be conducted using methods and operations of the invention. The invention operates to isolate small numbers of entities for detection, identification, and/or analysis. For instance, in Example 3, prior to the addition of PCR reagents (i.e., after subsample distribution into the wells), target and non-target molecules will be distributed in very small volumes according to the laws of probability. Thus, most wells will have no target molecules,

some will have one, and very few will have more than one, assuming sufficient wells exist in the device. The results of applying methods of the invention to isolation of subsamples containing mutant DNA fragments are illustrated below and in Figure 4. That Figure shows the distribution of DNA fragments (mutant and wild-type) in a 360 well plate after distribution as described above based upon a single stochastic process. Of the 360 wells shown, only 3 contain the single (mutant) molecule of interest. That molecule may then be amplified as described above or otherwise manipulated. The point, however, is that the presence of that single molecule was detected in a sample in which its presence may not have been detected using conventional methods.

Table 1 shows cumulative results of 500 separate fractionations in which a single biological sample is fractionated according to methods described above.

TABLE 1

Inputs:									
	Nominal number of Wild-Type Molecules				150				
	Nominal Mutant to Wild-Type Ratio				3%				
	Number of Wells				360				
	Number of experiments (1-500)				500				
Outputs Through 500 Runs:									
		Total Wild templates	Total mut templates	Wells with no template at all	No Mutant One Wild-Type	No Mutant More than one Wild-Type	One Mutant No Wild-Type	One Mutant One Wild-Type	One Mutant More than one Wild-Type
	Average	149.59	4.65	234.36	97.60	23.41	3.05	1.26	0.30
	Std Dev	8.20	2.13	6.94	7.83	3.97	1.73	1.18	0.51

Out of 360 wells, about 234 had no template (i.e., no wild-type or mutant DNA), about 98 wells had only one wild-type fragment, and about 3 had one mutant fragment. Fragments in all wells are amplified in parallel, and the amplicons produced are identified using, for example, fluorescent markers. Methods as described above therefore allow detection of a low-frequency mutant fragment as well as wild-type fragments in a sample.

What is claimed is:

1. A device for fractionating a liquid sample, the device comprising:

A plurality of interconnected channels comprising

a plurality of vents for creating breaks in the channels, thereby to separate a liquid sample into subsamples; and

a plurality of valves for depositing subsamples into receptacles for analysis.

2. The device of claim 1 further comprising an inlet for introducing a liquid sample into the device.

3. The device of claim 2 further comprising a reservoir in fluid communication with said inlet and at least one of said channels.

4. The device of claim 2, wherein said plurality of interconnected channels comprises a primary sample flow channel in fluid communication with a plurality of secondary channels, each of said secondary channels comprising a series of alternating vents for creating a series of vacuum breaks and valves for depositing subsample created by said vacuum breaks.

5. The device of claim 2, wherein each member of said plurality of interconnected channels emanate from said inlet.

6. The device of claim 4, further comprising a purge channel in fluid communication with said primary sample flow channel, wherein a purge valve separates said purge channel from said sample flow channel.

7. The device of claim1, wherein said valves are pressure-activated valves.

8. The device of claim 7, wherein said valves are hydrophobic.

9. The device of claim 6, wherein said purge valve is activated at a pressure that is different than the pressure necessary to activate said valves.

10. The device of claim 1, wherein said channels are etched, machined, stamped, or embossed onto a substrate, and said vents comprise a porous sheet covering said substrate.

11. The device of claim 10, wherein said sheet is selected from the group consisting of a Zitex ® sheet and a Goretex® sheet.

12. The device of claim 10, further comprising a lid attached to said substrate for sealing said device.

13. The device of claim 10, further comprising a multi-well receptacle for receiving subsample from said device.

14. The device of claim 13, wherein said substrate comprises a plurality of apertures associated with said valves for depositing subsample into said receptacle.

15. The device of claim 4, further comprising additional main sample flow channels, each associated with at least one secondary channel.

16. A device for fractionating a liquid sample, the device comprising:
an inlet for introducing liquid sample into the device;

a reservoir in fluid communication with said inlet;

a main sample flow channel in fluid communication with said reservoir

a plurality of secondary channels, each in fluid communication with said main sample flow channel, and each comprising a series of bends;

a plurality of vents for creating vacuum breaks in said secondary channels;

a plurality of valves for depositing subsample into a receptacle; and

a purge channel in fluid communication with said main sample flow channel, said purge channel being separated from said main sample flow channel by a valve.

17. The device of claim 16, wherein said main sample flow channel is separated from said reservoir by a purge valve.

18. The device of claim 16, wherein said valves are pressure-activated valves.

19. The device of claim 18, wherein said valves are activated at different pressures.

20. The device of claim 17, wherein said purge valve is activated at a different pressure than said valves.

21. A method for fractionating a liquid sample, the method comprising the steps of:

introducing a liquid sample into a device according to claim 1;

applying pressure to said device, thereby to cause sample to move through the device; and

activating said vents, thereby to separate said sample into subsamples by creating a plurality of vacuum breaks.

22. The method of claim 21, further comprising the step of depositing said subsamples into a receptacle which receptacle can be either on the device or mounted in proximity to it.

23. The method of claim 22, further comprising the step of analyzing said subsamples.

24. The method of claim 21, wherein said applying and activating steps comprise rotating said device.

25. The method of claim 22, wherein said depositing step comprises rotating said device.

26. The method of claim 22, wherein said depositing step comprises applying pressure to liquid in said channels, thereby to activate said vents.

27. The method of claim 21, wherein said sample is a biological sample.

28. The method of claim 21, wherein said sample is a non-biological sample.

29. The method of claim 23, wherein said analyzing step comprises conducting one or more polymerase chain reaction.

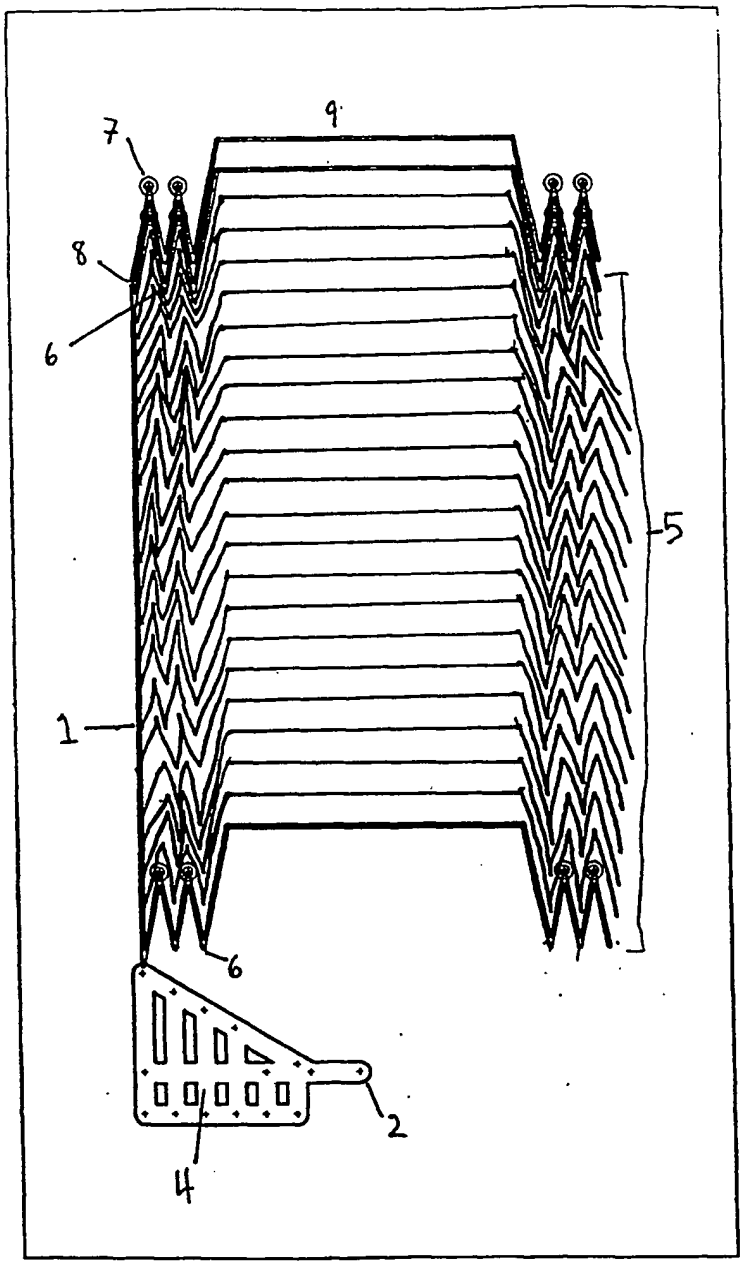

Fig. 1

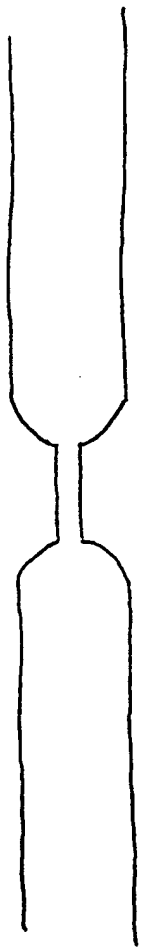

Fig. 2

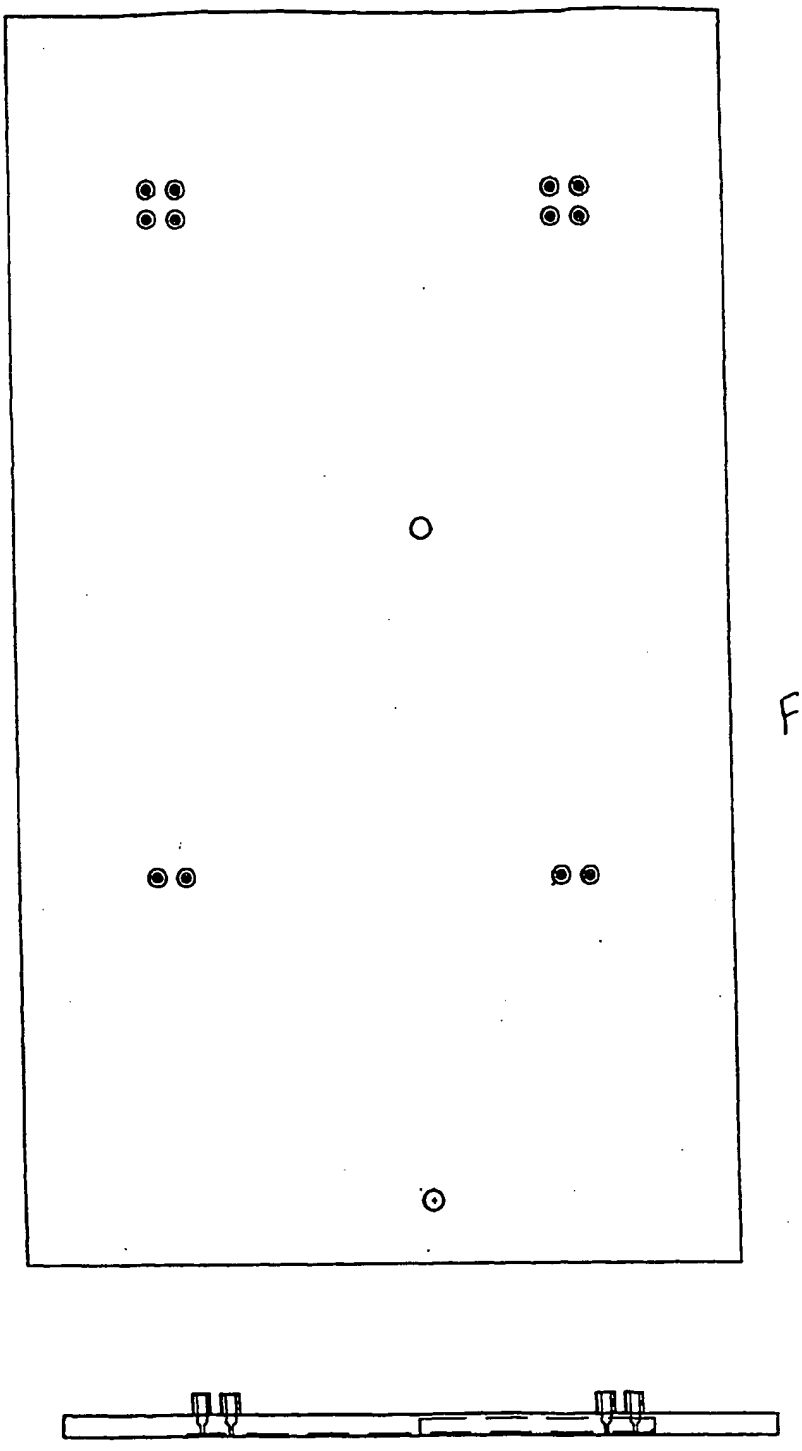

Fig. 3

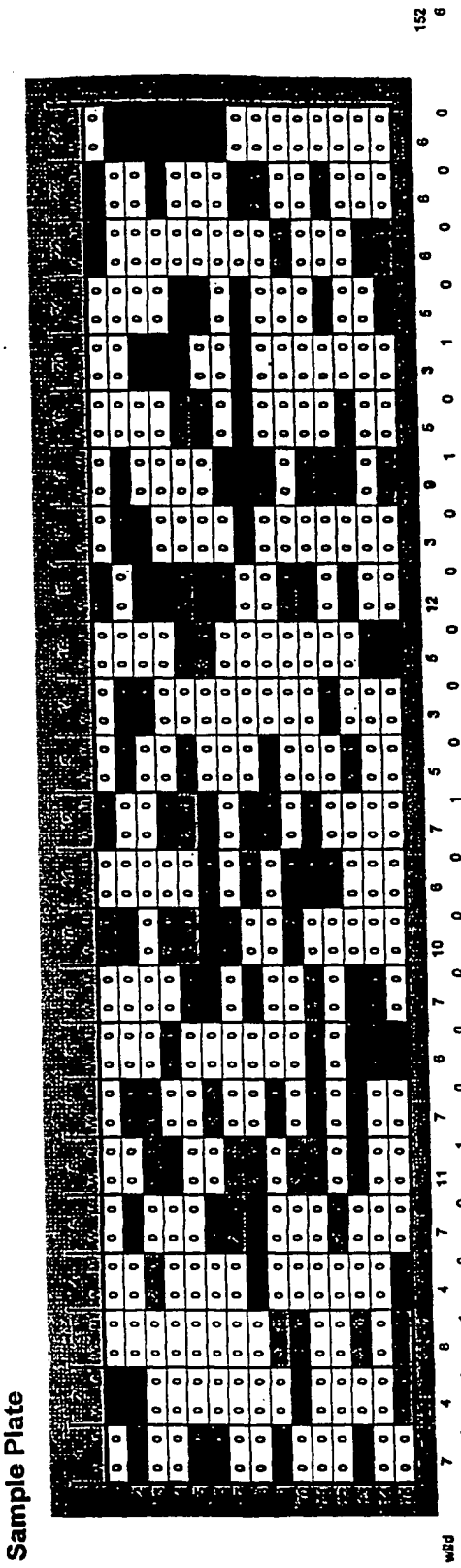

Figure 4